REMARKS

Claims 1, 11, 36, 42, 43, 82-88, 90-96 are pending in the instant application.

It is respectfully submitted that the present amendment presents no new issues or new matter and places this case in condition for allowance.

I. The Rejection of Claims 1, 11, 36, 42-43, 82-88, and 90-96 under 35 U.S.C. § 103

Claims 1, 11, 36, 42-43, 82-88, and 90-96 stand rejected under 35 U.S.C. § 103 as being unpatentable over Wilson *et al.* (*PNAS* 96: 12833-12838, 1999) in view of Cao *et al.* (*Mol. Microbiol.* 45: 1267-1276, 2002) for the reasons of record. This rejection is respectfully traversed for the reasons of record and further for the reasons stated below.

The Office asserts that it would have been prima facie obvious at the time of applicants' invention to apply the *Bacillus subtilis* strain used in the DNA hybridization microarrays of Cao *et al.* to the Wilson *et al.* method for determining the mode of action of an antimicrobial compound comprising detecting hybridization complexes and assigning a mode of action in order to obtain an antimicrobial mode of action for *B. subtilis* which is known to be resistant to known antimicrobial drugs.

Wilson *et al.* teach the use of DNA microarrays to characterize the global transcriptional response of *Mycobacterium tuberculosis* to isoniazid (INH) at concentrations of 0.2 µg or 1 µg of INH per ml, which are above the minimum inhibitory concentration of INH, *i.e.*, 0.02 µg of INH per ml (see Argyrou *et al.*, 2006, *Nature Structural & Molecular Biology* 13: 408-413, which cites Bernstein *et al.*, 1952, *Am. Rev. Tuberc.* 65: 357-364 and Youatt, 1969, *Am. Rev. Respir. Dis.* 99: 729-749). Wilson *et al.* use isoniazid (INH) at concentrations above the minimum inhibitory concentration of INH.

Cao *et al.* teach the use of DNA microarrays to characterize the global transcriptional response of *Bacillus subtilis* to vancomycin at concentrations 10X the minimum inhibitory concentration (see page 1269, column 2, last paragraph). Cao *et al.* use vancomycin at concentrations 10X the minimum inhibitory concentration.

The Office states: "Wilson *et al.* teach concentrations of 0.2 µg or 1 µg of INH per ml, growth occurs and the bacteria is not killed by the INH-induced expression profiles" and thus "Wilson *et al.* meets the sub-inhibitory limitation of the claims." Applicants submit that the Office has mischaracterized Wilson *et al.* Wilson *et al.* state the following on page 12834 under "Growth and Drug Treatment of *M. tuberculosis* Strains" in the second paragraph:

> Cultures for experimental treatment were initiated by diluting a frozen stock inoculum 1:200 into fresh 7H9 media in vented, screw-cap, tissue culture flasks and grown to early log phase (0.15-0.3 OD_{600}) with shaking (80 rpm) in a 5% CO_2 atmosphere at 37°C. Drug treatment was begun by adding filtered stock solutions of INH (1 mg/ml, Sigma) or ethionamide (25 mg/ml, Sigma) to achieve the following final concentrations: 0.2 µg/ml or 1 µg/ml for INH and 5 µg/ml or 20 µg/ml for ethionamide. Upon completion of the predetermined duration of drug treatment, the bacteria were harvested by centrifugation, and the pellets were rapidly frozen in crushed dry ice and stored at -80°C for RNA isolation.

Consequently, Wilson *et al.* first grow the *M. tuberculosis* strain to early log phase before the addition of INH at a concentration of 0.2 µg or 1 µg per ml. It appears the Office has incorrectly concluded that INH was added at the beginning of growth and thus present during growth to early log phase, which it was not.

The Office also states: "Applicants write that Wilson *et al.* teach concentrations that are above the MIC. In order for the cultures to result in growth and not die, the concentrations must be above the MIC. It is agreed that Wilson et al., teach concentrations above the Minimum Inhibitory Concentration amount. Moreover, all of the claims require that the concentration be above the MIC amount, in order to assign a mode of action for that antimicrobial compound." Applicants submit that the Office's statements are incorrect. The instant claims state "sub-inhibitory amount". Wilson *et al.* use isoniazid (INH) at concentrations **above** the minimum inhibitory concentration of INH. Applicants use concentrations **below** the minimum inhibitory concentration. Applicants submit that the Office's statement "for the cultures to result in growth and not die, the concentrations must be above the MIC" is technically wrong and not supported by Wilson *et al*. The growth aspect is addressed by Applicants above.

The Office also states: "Wilson *et al.* teach concentrations of 0.2 µg or 1 µg of INH per ml, which are **below** [emphasis added] the minimum inhibitory concentration of INH, *i.e.*, 0.02 µg of INH per ml; thereby using concentrations that result in growth, while also meeting the limitations of the claims." Applicants submit that the Office's statement is incorrect. Since the minimum inhibitory concentration of INH is 0.02 µg of INH per ml, the use of concentrations of 0.2 µg or 1 µg of INH per ml are **above** the minimum inhibitory concentration. The growth aspect is addressed by Applicants above.

The Office also states: "0.02 µg of INH merely respects an example of a subinhibitory amount". Applicants submit that the Office's statement is incorrect. The minimum inhibitory concentration of INH is 0.02 µg of INH per ml. Consequently, 0.02 µg of INH per ml is **NOT** an example of a subinhibitory amount.

The Office also states: "[I]t is the Office's position that where the general conditions of a

claim are disclosed in the prior art, it is not inventive to discover workable ranges by routine experimentation. *In re Aller*, 220 F.2d 454, 456, 105 USPQ 233, 235 (CCPA 1955). In this case, the prior art sets forth the use of subinhibitory amounts when detecting hybridization complexes ..." As Applicants state above, Wilson *et al.* and Cao *et al.* use concentrations above the minimum inhibitory concentration and do not teach or suggest the use of subinhibitory amounts.

As the record shows, Applicants submit that the Office's reliance on *In re Aller* is misplaced. *In re Aller* held that "it's obvious to routinely experiment and optimize" according to the facts of *In re Aller*. The facts in *In re Aller* involve treatment of isopropyl benzene hydroperoxide (or similar organic peroxides) with sulphuric acid, wherein the hydroperoxide is decomposed into phenol and acetone (or other ketones). The process was identical with that of the prior art, except that the claims specified lower temperatures and higher sulphuric acid concentrations than shown in the prior art. The claimed process in *In re Aller* resulted from experimentally varying the different factors of the prior art process to optimize the reaction conditions and there was no evidence to indicate that the reported increase in yields was a difference in kind and not of degree.

In the instant application, the facts are different. Applicants have not optimized the inhibitory range of an antimicrobial compound. Wilson *et al.* teach the use of isoniazid (INH) at concentrations of 0.2 µg or 1 µg of INH per ml, which are above the minimum inhibitory concentration of INH. Cao *et al.* teach the use of vancomycin at concentrations 10X the minimum inhibitory concentration. Wilson *et al.* in view of Cao *et al.* teach or suggest the use of microarray hybridization for determining gene expression in response to antimicrobial compound concentrations above their minimum inhibitory concentration of INH. One skilled in the art would recognize that optimization of the inhibitory range of an antimicrobial compound would involve concentrations above the minimum inhibitory concentration, not sub-inhibitory concentrations. Applicants have not optimized the inhibitory range of an antimicrobial compound, but rather use sub-inhibitory concentrations, which do not fall within or overlap concentrations above the minimum inhibitory concentration. Consequently, the facts of Applicants' invention are distinguishable over the facts of *In re Aller*. Applicants submit that *In re Aller* is not relevant to the instant application.

Applicants submit that the claimed methods produce unexpected results that are different in kind and not merely in degree from the results in the cited references by utilizing sub-inhibitory amounts of an antimicrobial compound. Applicants have shown that the use of sub-inhibitory amounts of an antimicrobial compound result in the ability to more readily identify

primary effects of the antimicrobial compound on genes of a bacterial cell and reduce secondary effects on other genes that can result from using high inhibitor concentrations of the compound. The use of sub-inhibitory concentrations consequently slows the action of the compounds, and limits the expression of genes that are correlated to secondary effects, allowing a predominance of expressed nucleic acids that correlate with the activity of the antimicrobial compound, which is related directly, and primarily, with its mode of action on the cell. In contrast, gene expression responses to concentrations of an inhibitor above its minimum inhibitory concentration cause a broader effect on cellular processes by the inhibition of secondary targets within the cell, as well as by downstream effects that result from inhibition of the primary target, thereby giving much more complex response patterns. Applicants' submit that their results exhibit a superior advantage that a person skilled in the art would have found surprising and unexpected.

For the foregoing reasons, Applicants submit that the rejections under 35 U.S.C. § 103 have been overcome and respectfully request reconsideration and withdrawal of the rejections.

II. Conclusion

In view of the above, it is respectfully submitted that all claims are in condition for allowance. Early action to that end is respectfully requested. The Examiner is hereby invited to contact the undersigned by telephone if there are any questions concerning this amendment or application.

Respectfully submitted,

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